SPLEEN CELL PROLIFERATION IN RESPONSE TO HOMOLOGOUS ANTIGENS STUDIED IN CONGENIC RESISTANT STRAINS OF MICE*, ‡

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(Received for publication 13 December 1965)

A marked proliferative response occurs when spleen cell suspensions from two different strains of inbred mice are incubated together (1).[1] 2 to 4% of the total cell population are rapidly dividing by 48 hr and the size of the response can be readily determined by measurement of the incorporation of radioactive thymidine during the period 24 to 48 hr after the start of incubation.

The biological significance of the response has not been conclusively established, but it is probable that the proliferation represents the first stage in an immunological reaction to homologous tissue antigens (1, 2). The response is undoubtedly dependent on recognition of some phenotypic expression of the genetic differences between the two cell populations.

In a previous study it was shown that proliferative responses were obtained in almost all of 21 combinations of seven strains of inbred mice (1). The size of the response varied over a considerable range and was marginal in 2 cases.

The seven strains used differed from one another at several different genetic loci and were, therefore, not suitable for more detailed analysis.

The purpose of the present work is to define how small a genetic difference will produce a measurable response. The responses between cell suspensions from a variety of congenic resistant strains[2] were measured. Measurable re-

* This is publication No. 146 from the Division of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla.

‡ This work was supported in part by grant AI-7007 from the United States Public Health Service and in part by a Dernham Scholarship of the American Cancer Society, California Division (No. D-100), from Scripps Clinic and Research Foundation, La Jolla.

[1] It was found that one of the strains of mice used in this study was wrongly designated. The correct designations and the revision of the discussion can be found at the end of this paper.

[2] "*Coisogenic strains* are defined as strains genetically identical one with another except for a difference at a single locus. True coisogenicity is achievable only when a mutation occurs in an inbred stock. An approximation to the coisogenic state can be produced by continued crossing of a gene from one stock onto the inbred and therefore isogenic background of another. It may be presumed in this case that the foreign gene is always accompanied by and is part of a foreign chromosome segment. Other contaminant genes may also be present, but

sponses were obtained in 9 out of 12 cases where the strain pairs differed at the H-2 locus. No responses were observed between cells that differed at "weaker" histocompatibility loci, or between cells from male and female mice of the C57BL/6 strain.

Materials and Methods

Inbred and congenic resistant strains of mice (Table I) were obtained from the Jackson Laboratory, Bar Harbor, Maine, and were used when 8 to 12 wk old. Spleen cell suspensions were prepared and incubated in a modified Eagle's MEM suspension medium as previously described (1). In these experiments the concentration of mouse serum was reduced from 15 to 5%. All cultures contained a total of 3×10^7 cells in 2 ml of medium. Mixed cell suspensions contained equal numbers of cells from the two strains. The proliferative response was measured by the incorporation of tritiated thymidine during the period 24 to 48 hr after the start of incubation. At the end of that time, the cells were harvested, washed, dissolved in hyamine, and counted in a liquid scintillation counter as previously described (1).

The data reported represent the means of triplicate determinations.

RESULTS

Cell suspensions were prepared from the spleens of several mice of the same inbred strain. They were incubated alone or mixed in equal proportions with similar cell suspensions prepared from different inbred strains. In each case the rate of DNA synthesis was measured by determining the uptake of labeled thymidine during a standard incubation period. The strains of mice used in this study are listed in Table I. In the experiments performed, mixed cell suspensions were made between congenic resistant pairs of mice which differed at a single locus which controlled a known histocompatibility antigen (3). A stimulatory response was recognized when the incorporation of thymidine into the mixed culture was greater than the average of the incorporation into the two separate cultures. The results are expressed as the ratio of the homologous "mixed" culture to the isologous controls.

Table II shows the results obtained in 12 cases where congenic resistant strains differing at the H-2 locus were mixed. For this study, four inbred partner strains, each having two congenic resistant strains, were used. The inbred partners were mixed with each of their congenic resistant strains and, in addition, the two congenic resistant strains themselves were mixed. The H-2 loci of each pair of suspensions is listed. The results of two separate experiments are recorded. Further data on some of the mixes is contained in Table III. It

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the length of the foreign chromosome segment and the number of contaminant genes will decrease as the number of matings of the specified foreign gene to the inbred parent strain increases.

A strain derived in this fashion which approximates but may never fully achieve the true coisogenic state is referred to as a *congenic strain.* The inbred strain to which repeated crosses have been made is the *inbred partner.* A congenic strain and its inbred partner are referred to as a *congenic pair.* If the foreign gene is the congenic partner is a histocompatibility gene, so that the members of the congenic pair mutually resist transplants of each other's tissues, the congenic strain is a *congenic resistant* or *CR strain* and the pair a *congenic resistant* or *CR pair,*" Snell (6).

can be seen that there was a stimulation of DNA synthesis in 9 cases out of 12. In 3 cases, all involving AKR mice, the response was minimal. Two reference mixes were included in each experiment (lines 13 and 14) of strain combinations which had multiple genetic differences.

TABLE I

Inbred and Congenic Resistant (CR) Strains

	H-1	H-2	H-7	H-8	H-9
Inbred partner					
C57BL/10ScSn	c	b	a	a	a
CR lines					
B10.C3H (40 NX)	*a*	b	a	a	a
B10.A	c	*a*	a	a	a
B10.D2-new	c	*d*	a	a	a
B10.C (47 N)	c	b	*b*	a	a
B10.D2 (57 N)	c	b	a	*b*	a
B10.C (45 N)	c	b	a	a	*b*
Inbred partner					
C3H/DiSn		k			
CR lines					
C3H.SW		b			
C3H.NB		p			
Inbred partner					
AKR/JSn		k′			
CR lines					
AKR.K		a			
AKR.M		m			
Inbred partner					
A/WySn		a			
CR lines					
A.BY		b			
A.CA		f			
AKR/J		k			
BALB/cJ		d			

In a second series of experiments (Table III), the responses of pairs of strains differing at H-1, 2, 7, 8, and 9 loci were measured. Positive responses were obtained with strain pairs differing at the H-2 locus as before, but the stimulation with mixes involving the H-1, 7, 8, and 9 were not statistically significant. Reference mixes (lines 7 and 8) were again included in each experiment.

Finally, (Table IV), it was found that no statistically significant response was

obtained when male and female cells of the C57BL/6 strain were mixed. Positive responses of C57BL/6 female cells to B6D2F$_1$ were observed in each experiment (line 2).

TABLE II

Responses between Cells of CR Lines Differing at the H-2 Locus

Strain combination	H-2 loci	Ratio thymidine uptake: Homologous mix/isologous controls	
		Experiment 829	Experiment 882
C57BL/10ScSn + B10.A	b + a	3.5	3.3
C57BL/10ScSn + B10.D2 (new)	b + d	3.2	3.4
B10.A + B10.D2 (new)	a + d	2.6	2.5
C3H/DiSn + C3H.SW	k + b	3.6	3.0
C3H/DiSn + C3H.NB	k + p	3.5	3.0
C3H.SW + C3H.NB	b + p	4.5	4.5
AKR/JSn + AKR.K	k + a	1.9	1.6
AKR/JSn + AKR.M	k + m	1.6	1.4
AKR.K + AKR.M	a + m	1.3	1.1
A/Wy.Sn + A.BY	a + b	3.1	4.0
A/Wy.Sn + A.CA	a + f	4.5	2.3
A.BY + A.CA	b + f	4.1	2.2
B57BL/10ScSn + AKR/JSn	b + k*	6.5	5.5
C3H/DiSn + A/WySn	k + a*	1.4	1.1

Spleen cell suspensions from each pair of inbred strains were incubated separately (isologous controls) or mixed in equal proportions (homologous mixed). The thymidine uptake of each suspension was measured in the period 24 to 48 hr after the start of incubation. The results are expressed as the ratio of the thymidine incorporation into the homologous mixed cell suspensions to the average incorporation into the two isologous control cell suspensions.

* Plus multiple other differences.

DISCUSSION

The results show that proliferative responses are obtained in many cases when spleen cell suspensions from two strains of mice differing at a single gene locus are mixed, when that locus controls an H-2 histocompatibility locus. These responses were of the same order of magnitude as the response obtained with strain combinations where there were multiple genetic differences. No measurable responses were obtained under these conditions when the genetic difference involved so-called weak histocompatibility antigens. These two observations have been taken together as providing strong circumstantial evidence

that the response is a measure of an immunological response. Conclusive evidence, however, is still lacking, as has been previously discussed (1).

It should be noted that the congenic resistant strains used in this study represent only an approximation to the coisogenic state in which two strains truly

TABLE III

Responses between Cells of CR Lines Differing at H-2 and Weak Histocompatibility Loci

Strain combination	H loci	Ratio thymidine utpake		
		Homologous mix/isologous controls		
		Experiment 844	Experiment 848	Experiment 849
C57BL/10ScSn + B10.C3H (40NX)	1c + 1a	1.0	1.1	0.9
C57BL/10ScSn + B10.A	2b + 2a	3.8	3.6	2.7
C57BL/10ScSn + B10.D2 (new)	2b + 2d	3.1	5.8	2.2
C57BL/10ScSn + B10.C (47N)	7a + 7b	0.9	1.1	0.9
C57BL/10ScSn + B10.D2 (57N)	8a + 8b	0.8	1.9	0.9
C57BL/10ScSn + B10.C (45N)	9a + 9b	0.9	1.8	0.9
C57BL/10ScSn + AKR/J	2b + 2k*	5.6	5.9	6.8
C57BL/10ScSn + BALB/c	2b + 2d*	4.3	5.3	5.9

See footnote to Table II.

* Plus multiple other differences.

TABLE IV

Responses between Cells of Male and Female Mice of C57BL/6 Strain

Strain combination	Ratio thymidine uptake					
	Homologous mix/isologous controls					
	Experiment No.					
	785	794	796	818	819	825
C57BL/6 female + B6 male	1.1	1.3	1.6	1.1	1.3	1.0
C57BL/6 female + B6D2F1	6.5	8.2	8.2	3.6	4.5	2.3

See footnote to Table II.

differ by only a single gene (3). It cannot be excluded that the responses measured were dependent on minor genetic differences other than the specific histocompatibility locus for which the strain pair had been selected, although this is considered unlikely.

The H-2 loci represent a series of alloantigenic specificities controlled by closely linked genes (4). Many of these are common to several different H-2 loci.

In any given mix each partner will lack components present in the other and the response measured must presumably represent the sum of these two separate responses. It would seem possible to investigate the relative importance of the components using F_1 hybrids (1), but this was not attempted in this study.

In some mixes there were numerous alloantigenic specificity differences between the two strains and in others only a few. For example, there were 14 between C3H/DiSn (H-2K) and C3H. SW (H-2b) (Table II), while in others there were relatively few, for example, five between B10.A (H-2a) and B10.D2 (H-2d). There was no obvious correlation between the number of recognized differences and the size of the response. In many cases the number of differences has not yet been defined.

It may be significant that the three cases where H-2 differences did not give rise to significant responses all occurred in AKR mice. It is possible that the AKR mouse is genetically deficient in its ability to mount an immunological response to some histocompatibility antigens compared with other inbred strains. It is conceivable that such a deficiency could be correlated with a preleukemic state (5). This possibility is under current investigation comparing the responses with the same H-2 difference against different genetic backgrounds.

In earlier experiments, it had been found that minimal responses were obtained when cells were mixed with C3H A/J (1). In this case there is a difference at the H-2 locus. The reason for the apparent discrepancy between reactivity in this system and skin rejection reactivity is not known.

The lack of response in mixes where the difference lies in weak histocompatibility loci correlates with the longer (sometimes greater than 200 days) skin graft rejection times in these strain combinations (6).

The Y chromosome of the male is, or carries with it, a weak histocompatibility antigen that results in the rejection of male skin grafts on females in a certain percentage of mice (7, 8). It was of interest, however, that no response could be obtained in C57BL/6 female mice against male cells even with mice that had rejected three consecutive male skin grafts (9).

Although no responses were obtained in strain combinations where there was a single difference at a weak histocompatibility locus, it had been observed earlier (1) that responses could be obtained between DBA/2 and BALB/c, between AKR and C3H, between DBA/2 and B10.D2, and between BALB/c and B10.D2. In each of these strain pairs there is no H-2 difference and the response may be presumed to depend on the presence of multiple "non-H-2" differences.

SUMMARY

The proliferative responses obtained when spleen cell suspensions from two different inbred strains of mice were mixed were investigated further using congenic resistant strain pairs.

Strong responses were obtained in 9 cases out of 12 where the two strains

differed at a single gene locus controlling an H-2 histocompatibility antigen. No responses were obtained where the difference occurred at loci controlling weak histocompatibility antigens.

These findings have been taken to provide additional circumstantial evidence that the response represents an in vitro homograft reaction to homologous tissue antigens.

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Addendum.—An error was made in the designation of one of the strains used in a previous study:

> Dutton, R. W., Further studies of the stimulation of DNA synthesis in cul tures of spleen cell suspensions by homologous cells in inbred strains of mice and rats, *J. Exp. Med.*, 1965, **122,** 759.

Strain I., designated as C57BL/10ScSn (new), was really B10.D2 (new). This strain is an H-2d, while C57BL/10ScSn is H-2b.

Thus, six changes are required in the H-2 loci indicated in Fig. 2:

1 + 6 now represents d + b
2 + 1 now represents d + d
3 + 1 now represents d + d
4 + 1 now represents k + d
5 + 1 now represents k + d
1 + 7 now represents d + a

These changes require some alteration in the discussion of the results as follows: The combinations 2 + 1 and 3 + 1 now represent mixes involving no difference at the H-2 locus and the fact that the response between 3 and 1 was low is no longer surprising. The 4 combinations involving no H-2 difference now become more clearly lower than the rest.